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YidC – an evolutionary conserved device for the assembly of energy-transducing membrane protein complexes

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Members of the YidC/Oxa1/Alb3 membrane protein family are multifunctional mediators of membrane protein integration, folding and assembly into large complexes. Their evolutionary conserved and physiologically important role appears to relate to the assembly of major energy-transducing membrane protein complexes.

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Introduction

Many mechanistic aspects of intricate biochemical reactions mediated by large membrane protein complexes, such as respiratory electron transport or ion gradient-driven ATP synthesis have been elucidated during the past few years. However, the membrane integration and assembly of these large membrane protein complexes is far from understood.

All primary energy-transducing membranes contain a member of the cytochrome oxidase biogenesis (Oxa) membrane protein family [1,2]. In the inner membrane of mitochondria, Oxa1p mediates the integration of membrane proteins from the matrix side of the membrane. Known substrates of Oxa1p include mitochondrially encoded respiratory chain complex subunits, such as Cox2p, as well as nuclear encoded proteins such as Oxa1 itself [3–5]. In the chloroplast thylakoid membrane, the Alb3 protein is required for the integration of light-harvesting complex proteins (LHCPs) [6]. Bacterial cytoplasmic membranes contain one or more family members that appear to have multiple functions in the biogenesis of membrane proteins. The best-studied example is the YidC protein from the Gram-negative bacterium *Escherichia coli*.

In this review, we discuss the recent insights in the mechanism by which YidC facilitates membrane insertion.

Sec-dependent membrane protein integration

In *E. coli*, most membrane proteins are targeted to the SecYEG translocase via the evolutionary conserved SRP (signal recognition particle) pathway [7]. Translocation of hydrophilic polypeptide domains depends on the ATP-driven motor protein SecA [8–12]. For many membrane proteins the proton motive force (PMF) across the cytoplasmic membrane was shown to be required for insertion and assembly [12–18]. Crosslinking studies have shown that YidC is in close vicinity to transmembrane segments (TMS) of nascent Sec-dependent membrane proteins, such as the cell division protein FtsQ, leader peptidase (Lep) and mannitol permease, during their co-translational insertion [10,11,19–25]. A part of the cellular YidC appears to be physically associated with the Sec translocase [20,26]. Therefore, it was proposed that YidC mediates the lateral release of transmembrane segments from the SecYEG translocase. Several functional studies, however, indicate that YidC is not essential for their membrane insertion. First, *in vivo* depletion of YidC has little effect on the membrane insertion of the monotopic membrane protein FtsQ [10], while its effect on Lep insertion appears to be more pronounced but not critical [21]. Second, a recent *in vitro* study demonstrated that the membrane insertion of FtsQ could be reconstituted with proteoliposomes containing only SecYEG [12]. In this system, the hydrophilic carboxy terminal domain is translocated into the proteoliposome lumen, the TMS of FtsQ was shown to be stably integrated into the lipid bilayer in the absence of YidC. It is still possible that *in vivo* YidC is involved in the hydrophobic partitioning of Sec-dependent TMSs, but at least for FtsQ, it seems that such effects at the most relate to the kinetics of the reaction. Membrane proteins or even their specific TMSs may differ in their YidC requirement, but the features of such TMSs that determine this dependence remain to be elucidated. In addition, YidC seems to play a chaperone role in membrane protein assembly *in vivo*. Experimental support for this hypothesis comes from a recent study by Nagamori *et al.* [27••], who have shown that YidC is required for the correct folding of the polytopic membrane protein LacY, while it is dispensable for its membrane integration per se.

Whereas most studies indicate a role of YidC in membrane protein insertion, Fröderberg *et al.* [28] recently

presented evidence for a role of YidC in the Sec-dependent export of lipoproteins. YidC depletion inhibits the translocation of murein lipoprotein (Lpp) and bacteriocin release protein (Brp) *in vivo* and nascent chains of both proteins crosslink to the SRP protein Ffh, SecA, SecY but also to YidC. Since this crosslinking pattern is observed with many nascent membrane proteins, the specific role of YidC in lipoprotein secretion needs to be investigated in more detail.

Sec-independent membrane protein integration

In 2000, Samuelson *et al.* [21] observed that the Sec-independent insertion of the phage protein M13 procoat into the inner membrane of *E. coli* strictly requires YidC *in vivo*. M13 procoat membrane insertion was long believed to be a spontaneous event and a wealth of literature has been published that underscored this hypothesis. M13 procoat consists of two antiparallel TMSs; the first represents a cleavable signal peptide, while the second is a structural unit of the final phage coat. Translocation of the connection loop to the periplasm requires the PMF. As the depletion of YidC was shown to induce strong defects in PMF generation [29••] (see below), this raised the question, whether the effect of YidC depletion on M13 procoat insertion could be indirect. However, PMF-independent point mutants of M13 procoat appear to require YidC for efficient membrane insertion *in vivo* [30] and temperature-sensitive variants of YidC exhibit the M13 procoat insertion defect shortly after a shift to the non-permissive temperature [31], which is earlier than the PMF defects observed upon YidC depletion. This indicates that YidC mediates the membrane insertion of M13 procoat *in vivo*.

Chen *et al.* [32] further demonstrated that a second Sec-independent phage protein, Pf3 coat, requires YidC for membrane insertion both, *in vivo* and *in vitro*. A direct interaction between YidC and Pf3 coat was demonstrated by crosslinking. Pf3 coat is a polypeptide of only 44 amino acids that spans the membrane once with the N-terminus translocated to the periplasm. Therefore, it resembles the second TMS of M13 procoat. Insertion of Pf3 coat is also strongly dependent on the PMF. A recent study by Serek *et al.* [33] showed that the membrane insertion of Pf3 can be reconstituted with proteoliposomes containing only YidC.

What might be the mechanistic role of YidC in Sec-independent membrane protein insertion? For M13 it was shown, that both hydrophobic domains are essential for the translocation of the central loop indicating that an interaction between the two TMSs facilitates membrane insertion [34]. This helical hairpin formation is required because of the high abundance of negative charges in the loop that need to be translocated [35]. Interestingly, although the negative charges acquire a topogenic character in the presence of a PMF, the synergistic insertion of the two TMSs by hydrophobic interactions alone can drive the translocation [36]. Therefore, it appears that hydrophobic forces and the PMF contribute independently to membrane insertion and that both parameters can at least partly compensate a defect or reduction in the other. For Pf3 coat, a somewhat different scenario seems to exist as the protein contains only one TMS. Mutations in YidC have been identified that specifically affect either M13 procoat or Pf3 coat insertion [30]. The negatively charged residues in the amino terminus of Pf3 coat appear to move electrophoretically across the membrane as this process is driven by the PMF [16]. A mutant Pf3 coat protein (Pf3-4N) that lacks charged residues fails to insert into the membrane [17]. However, when the hydrophobicity of the TMS is increased (Pf3-4N3L), hydrophobic forces drive the spontaneous membrane insertion without topological control [17]. Pf3-4N3L does not require YidC for insertion [33]. These observations confirm the synergistic action of the PMF and hydrophobic forces as described for M13 procoat. However, the interaction of YidC with the Pf3 coat nascent chains is independent of the PMF [32], which supports the notion that YidC and the PMF act on different steps of the insertion reaction.

The data on M13 procoat as well as Pf3 coat strongly suggests that the function of YidC in membrane protein integration is to facilitate the hydrophobic interaction of TMSs with the membrane lipids. It seems likely that YidC provides a special amphiphilic surface that lowers the activation energy of the hydrophobic partitioning event for instance by overcoming the repulsion of the hydrophobic protein segments by polar lipid head groups. In addition, YidC might shield polar residues on their way through the hydrocarbon core of the membrane and promote the folding of hydrophobic regions within a membrane protein into its transmembrane conformation. As in the case of the M13 procoat, this process might involve helical hairpin formation.

Physiological role of YidC

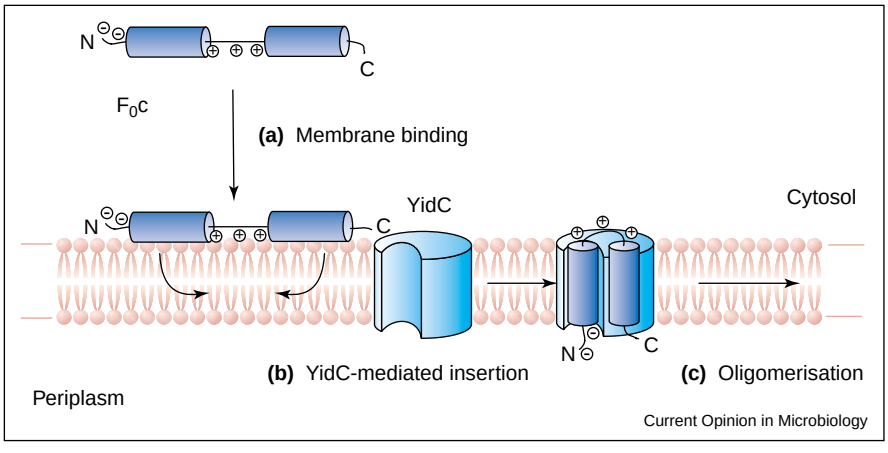
As discussed, YidC probably plays a kinetic role in the process of Sec-dependent membrane protein biogenesis and might act as a chaperone in the organization of helix–helix-interactions without being absolutely required for the insertion of Sec-dependent substrate proteins. In addition, M13 procoat and Pf3 coat strictly depend on YidC for membrane insertion, but these phage proteins do not represent authentic *E. coli* substrates nor are they essential for *E. coli* viability. This raised the question of whether YidC is an essential protein in *E. coli*? A physiological study using a YidC depletion strain showed that YidC depletion causes a strong induction of the membrane stress protein PspA [29••]. PspA induction is correlated with an impairment of the PMF. Further studies revealed that inner membrane vesicles prepared from YidC-depleted cells have a defect to generate a PMF

with both ATP and oxidizable substrates like NADH [29••]. The former correlates with decreased levels of functional F_1F_0 ATP synthase. In particular, the amount of the small, ring-forming rotor subunit c (F_0c) of the membrane-integral F_0 subcomplex is strongly reduced by YidC depletion [29••]. The reduced respiratory capacity is caused by decreased levels of cytochrome *o* oxidase complex from which the amount of the ubiquinol-binding subunit A (CyoA) in the membrane is severely reduced under YidC-depleting conditions [29••]. Since these two enzymes are the key energy-transducing protein complexes of aerobically grown *E. coli*, it seems that the essential role of YidC for *E. coli* viability is related to the biogenesis of these complexes. Yi *et al.* [37] reported that F_1F_0 ATP synthase subunits F_0c as well as F_0 subunit a (F_0a) and the Sec translocase subunit SecE require YidC for membrane insertion *in vivo*. However, F_0a does not stably integrate into the membrane in the absence of F_0c [38], and therefore it is not clear if the insertion defect of F_0a is directly related to the loss of YidC. Also, the observed insertion defect of SecE has to be assessed critically. SecY cannot assemble stably into the membrane in the absence of SecE, and SecE is essential for the functioning of the Sec-translocase. A membrane insertion defect of SecE would imply a strong pleiotropic effect on protein secretion and membrane protein insertion in general. This is not observed in the YidC depletion strain.

The biogenesis of bacterial F_0c was recently studied in detail by van der Laan *et al.* [39••] F_0c has a molecular mass of 8.3 kDa and consists of two TMSs connected by a small polar loop that is exposed to the cytoplasm. Insertion of *in vitro* synthesized F_0c into YidC-depleted membranes was strongly reduced compared to wild-type membranes. Since F_0c insertion is independent of the PMF, the insertion defect seems directly related to a role of YidC (see Figure 1 for a putative mechanistic model). Importantly, YidC proteoliposomes mediate the efficient and topologically correct membrane insertion of F_0c, while the Sec translocase and the SRP targeting pathway are not required. Although there are conflicting reports on the SRP requirement for F_0c insertion *in vivo* [40,41], the *in vitro* data clearly demonstrate that the SRP pathway is not essential as F_0c insertion into YidC proteoliposomes is not affected by reducing the amounts of either SRP or the SRP receptor FtsY to levels that block the integration of known SRP-dependent substrates, such as FtsQ [39••]. Upon insertion into YidC proteoliposomes, F_0c assembles into large oligomeric complexes in an analogous manner as *in vivo*. These data demonstrate that YidC functions as a membrane protein insertase for the F_0c subunit, which represents the first discovered physiological substrate of this novel insertion pathway. The membrane insertion requirements of F_0c are very similar to that described for M13 and Pf3 procoats (see Figure 2 for a putative mechanistic model). Therefore, it seems that these phage coat proteins employ an essential host cell membrane protein biogenesis pathway to become inserted into the membrane, a prerequisite for phage particle assembly.

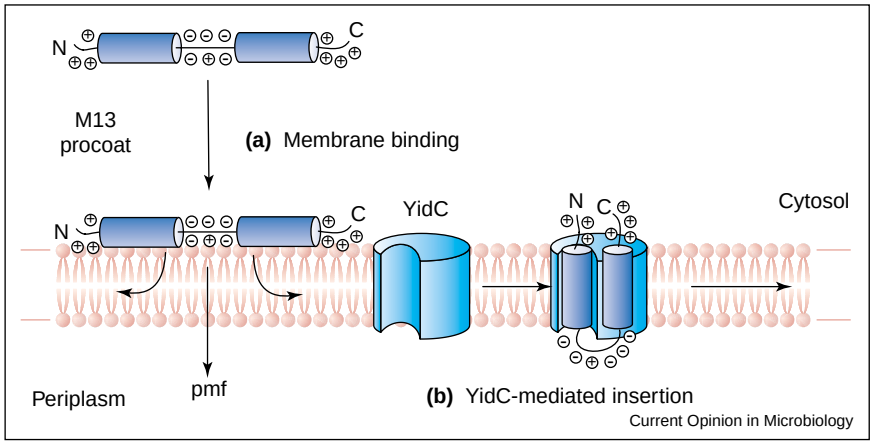
Ossenbuhl *et al.* [42••] recently demonstrated that the assembly of the structural core protein D1 into photosystem II in thylakoid membranes of the green algae *Chlamydomonas reinhardtii* is retarded in the absence of Alb3, while membrane integration per se is not affected. In the cyanobacterium *Synechocystis* PC668, deletion of the YidC homolog causes strong defects in the assembly

Figure 1



Model of the YidC-mediated membrane insertion of the F_0-subunit c. **(a)** F_0-subunit c initially binds to the membrane via electrostatic interactions. **(b)** Subsequently, YidC provides an amphiphilic surface that facilitates the hydrophobic partitioning of F_0c into the membrane and helical hairpin formation. **(c)**These hydrophobic interactions drive the translocation of the amino- and carboxy-terminal domains across the membrane. Positively charged amino acid residues anchor the hydrophilic loop that connects the two transmembrane segments at the cytosolic surface of the membrane. Next the F_0c subunits assemble into an oligomeric ring structure by a mechanism that is unknown.

Figure 2



Model of the YidC-mediated membrane insertion of M13 procoat. For the insertion of M13 procoat, the common principle of electrostatic membrane binding and hydrophobic partitioning facilitated by YidC is identical as for F_0c insertion (see Figure 1). However, the charge distribution in M13 procoat is inverted. Therefore, the amino- and carboxy-terminal domains are retained in the cytosol while the translocation of the negatively charged hydrophilic loops is strongly stimulated by the proton motive force (PMF). After insertion, procoat is processed by signal peptidase to yield the mature coat that consists of the carboxy-terminal transmembrane segment only.

of the photosystems [43]. These new observations together with the known assembly defects of ATP synthase and cytochrome c oxidase upon deletion of *OXA1* in yeast [44] suggest a general, evolutionary conserved role for Oxa family members in the biogenesis of energy-transducing membrane protein complexes.

Future perspectives

Many questions regarding the mechanistic role of YidC in membrane protein integration and assembly are still open. The mechanistic role of YidC in the Sec-dependent pathway remains unclear. Recently, a direct interaction of Alb3 with the chloroplast SecY has been demonstrated [45]. Mitochondrial inner membranes, however, lack a Sec-type translocase suggesting that only the Sec-independent function of the Oxa family members are evolutionary conserved. In addition, it is not understood how proteins are targeted to the independent YidC/Oxa1/Alb3 insertase. While most studies so far indicate that YidC substrates in bacteria do not require the SRP pathway, a direct interaction of chloroplast SRP/FtsY with Alb3 has been detected [46]. Oxa1 was recently shown to bind mitochondrial ribosomes, which suggest a co-translational insertion mechanism [47•,48•]. However, the C-terminal domain of Oxa1 required for this interaction is absent in YidC.

The main challenge will be to understand the mechanistic role of YidC/Alb3/Oxa1 in the insertion and assembly of large energy-transducing membrane protein complexes. For instance, how are other membrane-integral subunits of cytochrome *o* oxidase and F_1F_0 ATP synthase in *E. coli* inserted? An initial *in vivo* analysis suggests that subunits a and b of the F_1F_0 ATP synthase depend on the Sec translocase for membrane integration [40]. The YidC-dependent CyoA protein contains a large, carboxy-terminal periplasmic domain, which likely requires SecYEG and SecA for its correct localization. Therefore, it seems that YidC and SecYEG cooperate in the membrane insertion of CyoA, and this would define CyoA as another class of strictly YidC-dependent proteins next to the F_0c, M13 procoat and Pf3 coat, which require YidC alone.

Taken together, it appears that YidC, Alb3 and Oxa1 have multiple functions in the insertion, folding and assembly of membrane proteins. How they interact with their substrate proteins and how they organize protein–protein interactions within the membrane is currently under investigation.

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48. • Szyrach G, Ott M, Bonnefoy N, Neupert W, Herrmann JM: **Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria**. *EMBO J* 2003, **22**:6448-6457.
Around the same time as Jia *et al*. [47•], this paper also described the binding of mitochondrial ribosomes to the carboxy terminal domain of Oxa1. Furthermore, it demonstrated that the direct ribosome–Oxa1 interaction is crucial for the membrane integration of Cox2, a known Oxa1 substrate.